TWO-CENTER MECHANISM FOR THE OXIDATIVE DECARBOXYLATION OF PYRUVATE BY THE PYRUVATE DECARBOXYLATING COMPONENT OF THE PYRUVATE DEHYDROGENASE COMPLEX OF PIGEON BREAST MUSCLE

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1. Introduction

The oxidative decarboxylation of pyruvate by the pyruvate decarboxylating component of the pyruvate dehydrogenase complex (PDH, EC 1.2.4.1.) proceeds in the presence of hydrogen acceptors such as dichlorophenol indophenol (DCPIP) via enzyme bound 2-α-hydroxyethyl-thiamine pyrophosphate (HETPP [1]). With HETPP as substrate, acetate and TPP is formed by the pyruvate decarboxylating component in the presence of DCPIP [2]:

$$\text{HETPP} + \text{DCPIP} \xrightarrow{\text{PDH complex}} \text{Acetate} + \text{TPP} + \text{DCPIP}\cdot\text{H}_2$$

The degradation of pyruvate by the decarboxylase component, which is composed of two different subunits and has a $\alpha_2\beta_2$ structure [3], requires the presence of two cofactors TPP and Mg^{2+} which are reversibly bound to the enzyme.

In this paper it is shown that HETPP as substrate can be degraded by the pyruvate decarboxylating component in the absence of TPP and Mg ions. By means of a comparison of HETPP degradation and the rate of TPP dissociation from the pyruvate decarboxylating component it is shown that the oxidative decarboxylating of α-ketoacids by the decarboxylase component proceeds via a two-center mechanism.

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2. Materials and methods

The pyruvate decarboxylating component with spec. act. 0.29 U/mg has been obtained from PDH [4] by KBr treatment [5]. HETPP was synthesized and purified as in [6]. TPP (Merck), pyruvate (Sigma) and DCPIP (Sigma) were commercial products. All other chemicals were of p.a. degree.

Kinetic experiments, in the presence of DCPIP, were performed in 0.05 M potassium phosphate buffer, pH 7.0, at 600 nm and 25°C with a Hitachi-spectrophotometer, model 356.

3. Results and discussion

Degradation of HETPP by the pyruvate decarboxylating component takes place at saturation concentrations of HETPP and DCPIP at about the same rate as the degradation of pyruvate (table 1). This is in contrast to pyruvate decarboxylase of yeast (EC 4.1.1.1.)

Table 1
Influence of the cofactors TPP and Mg^{2+} on the activity of the pyruvate decarboxylating component of PDH with HETPP and pyruvate as substrates

TPP	Mg	Activity with	
		Pyruvate (%)	HETPP (%)
+	+	100	41
–	–	5	90
+	–	20–50[a]	90
–	+	5	92

[a] This value depends on the residual content of Mg^{2+} in the enzyme preparations

Concentrations: 0.05 M potassium phosphate buffer, pH 7.0, $3.3 \cdot 10^{-6}$ M TPP, $3.3 \cdot 10^{-4}$ M Mg^{2+}, 10^{-4} M DCPIP. 80 μg component in total vol. 3 ml (all values refer to pyruvate as substrate = 100%)

which shows no activity with HETPP as substrate [7].

The experiments listed in table 1 reveal that the cofactors TPP and Mg^{2+} are not required for the oxidation of HETPP by the pyruvate decarboxylating component. They show moreover an inhibition of the HETPP reaction by TPP in the presence of Mg^{2+}. These results suggest a different binding mechanism for TPP and HETPP in the active center(s) of the component. On the other hand the competitive type of inhibition of HETPP degradation by the component shown in fig.1 indicates that HETPP and TPP cannot be bound simultaneously in the active center. The estimation of the dissociation rate of TPP from the pyruvate decarboxylating component in a series of dilution experiments brought a clarification of these seemingly contradictory results. In these experiments the decrease of activity was measured after addition of 100 μl enzyme solution (containing 240 μg component, 1 nM TPP and 0.2 μM Mg^{2+} in 0.05 M potassium phosphate buffer pH 7.0) to 2.9 ml solution which contained 0.29 μM DCPIP and 4.8 μM pyruvate in the same buffer (fig.2). A possible effect of pyruvate on the dissociation rate, e.g., when the HETPP formed during the reaction should have a dissociation rate different from TPP was excluded in control experiments where pyruvate was added 2 s and 10 s after the dilution step.

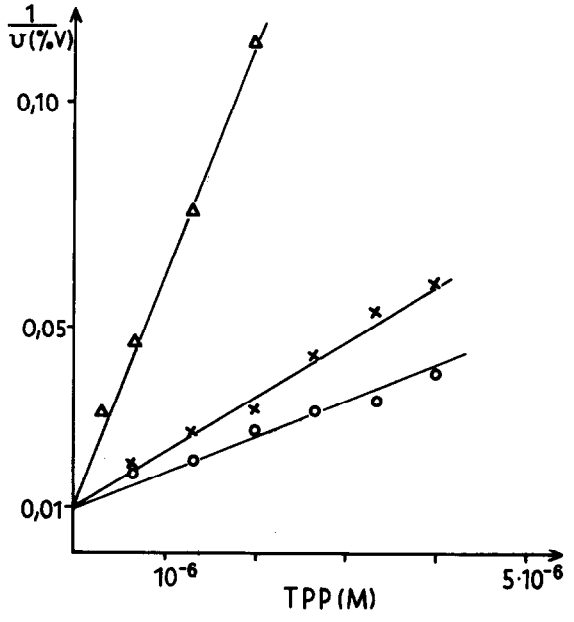

Fig.1. Dixon plot showing the competitive inhibition of HETPP degradation by TPP. 3.3×10^{-4} M Mg^{2+}, 10^{-4} M DCPIP HETPP: 2.2×10^{-4} (○), 7.35×10^{-5} M (×), 2.2×10^{-5} M (△).

From the ascent of the curves represented by the

$$\text{Slope} = \frac{k_d \cdot A_o}{2.303\,(A_o - A_e)}$$

a dissociation rate constant k_d 1.5 min^{-1} (A_o initial activity, A_e activity at equilibrium) was estimated. In the absence of substrate a value of about k_d ~3 min^{-1} was extrapolated (dashed curve in fig.2).

From the specific activity of the enzyme used in these experiments (0.29 U/mg) the overall catalytic activity was k_{cat} ~45 min^{-1}. Therefore, the dissociation of TPP or HETPP from the active centre cannot play a role in the reaction of the enzyme with pyruvate as substrate.

These results suggest a mechanism of oxidative decarboxylation by the pyruvate decarboxylating component of PDH which includes on the one hand different catalytic functions for the two subunits, and on the other hand the existence of a common active centre which may be formed in the contact region between the different subunits. One of these subunits catalyzes the decarboxylation of pyruvate to HETPP (e.g., the α-subunit) and requires TPP and Mg^{2+} ions. The second catalytic centre (which may be placed on the β-subunit) must be in direct contact with the

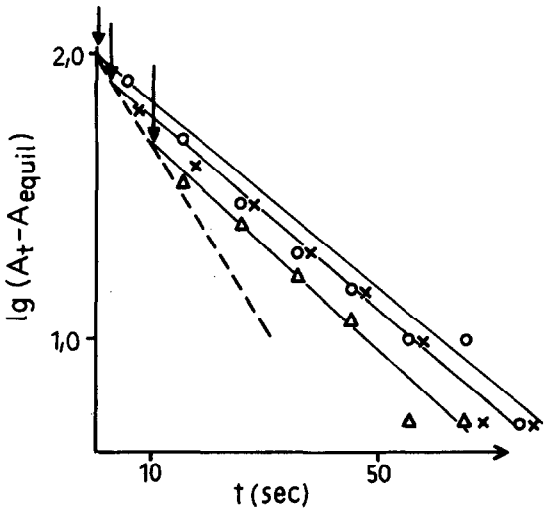

Fig.2. Log activity/time plot of coenzyme dissociation after dilution of the enzyme solution (see text). Addition of pyruvate immediately (○), after 2 s (×), after 10 s (△).

HETPP molecule which is formed in the first reaction. With this geometry of a common active center a dissociation step can be excluded and the competition between TPP and HETPP can be explained.

If HETPP is used as substrate the second catalytic activity can proceed without realizing a specific interaction with the α-subunit which gives related molecules such as TPP a chance to compete.

Recently a *ping–pong mechanism* has been proposed for HETPP degradation by the decarboxylase component in the presence of DCPIP [8]. This kinetic mechanism agrees with our results.

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